

ISOLATION AND CHARACTERIZATION OF A CHITINASE GENE FROM ENTOMOPATHOGENIC FUNGUS *VERTICILLIUM LECANII*

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Submitted: September 14, 2007; Returned to authors for corrections: November 23, 2007; Approved: January 23, 2008.

ABSTRACT

Entomopathogenic fungus *Verticillium lecanii* is a promising whitefly and aphid control agent. Chitinases secreted by this insect pathogen have considerable importance in the biological control of some insect pests. An endochitinase gene *Vlchit1* from the fungus was cloned and overexpressed in *Escherichia coli*. The *Vlchit1* gene not only contains an open reading frame (ORF) which encodes a protein of 423 amino acids (aa), but also is interrupted by three short introns. A homology modelling of Vlchit1 protein showed that the chitinase Vlchit1 has a $(\alpha/\beta)_8$ TIM barrel structure. Overexpression test and Enzymatic activity assay indicated that the Vlchit1 is a functional enzyme that can hydrolyze the chitin substrate, so the *Vlchit1* gene can service as a useful gene source for genetic manipulation leading to strain improvement of entomopathogenic fungi or constructing new transgenic plants with resistance to various fungal and insects pests.

Key words: Chitinase, Cloning, Overexpression, Homology modeling, *Verticillium lecanii*

INTRODUCTION

Verticillium lecanii is a well-known entomopathogenic fungus which has been commercialized for aphid and whitefly control (11). The host range of the species is wide and includes homopteran insects as well as a range of other arthropod groups. However, the full potential of this fungus as a mycoinsecticide is not yet exploited, mainly because the genetic and molecular basis of its pathogenesis in insects is not fully understood. The penetration of entomopathogenic fungi through the insect cuticle, which comprises mainly chitin fibrils embedded in a protein matrix, is essential for the infection. This key step in the insect infection occurs by a combination of mechanical pressure, via appressorium formation, and enzymatic degradation (2). During the penetration process, entomopathogenic fungi can produce several chitinases, some of which are important cuticle-degrading enzymes and act synergistically with proteases to hydrolyze insect cuticle (16). Chitinases have been implicated as pathogenicity determinants of entomopathogenic fungi (3). However, the roles that chitinases play in the infection process are still unclear (18). Overproduction

of *Bbchit* endochitinase can significantly enhance the virulence of *Beauveria bassiana* (5), suggesting that chitinase genes are candidates for genetic manipulation leading to virulence improvement of entomopathogenic fungi. Like other entomopathogenic fungi, *V. lecanii* produces chitinases that are able to degrade the cuticle of various insects effectively, and this aspect highlights the biocontrol potential of this fungus to insect pests (7, 8). Although chitinases of *V. lecanii* have considerable importance in the biological control of some insect pests, only two chitinase genes from this fungus have been reported till now. In order to better understand the role of chitinases in *V. lecanii* entomopathogenicity, we isolated and characterized the *Vlchit1* gene for further development of more efficient strains for the control of specific insect pests through genetic manipulation.

MATERIALS AND METHODS

Fungal and bacterial strains

V. lecanii strain Aa was originally isolated from a citrus whitefly *Dialeurodes citri* from a citrus-growing orchard in

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Fujian, China, in 2000. A single-spore isolate of Aa was stored in 20% glycerol at -80°C. Cultures were grown on potato dextrose agar (PDA) at 25°C with a daily cycle consisting of 15 h of light and 9 h of darkness. *E. coli* JM109 and TB1 were employed for DNA manipulation.

DNA and RNA preparation

V. lecanii mycelia were inoculated on PDA plate with cellophane. Cultures grown for 3 days were used for DNA extraction or transferred to induction medium (KCl 0.05% (w/v), MgSO₄ 0.05% (w/v), KH₂PO₄ 0.05% (w/v), Na₂HPO₄ 0.065% (w/v), Chitin 1% (w/v)) for chitinase induction. After 12 h induction, mycelia were harvested by filtration and washed with sterile distilled water three times, and then subjected to RNA extraction. DNA and RNA from *V. lecanii* were prepared as described by Reader and Broda (13) and Chomczynski and Sacchi (4), respectively. First strand cDNA for PCR amplification was synthesized by using AMV First Strand cDNA Synthesis Kit (BBI) in term of manufacturer's manual.

Gene cloning and sequencing

Five different fungal chitinase sequences (*B. bassiana*, accession number [AY145440](#), *Metarhizium anisopliae*, accession number [AF027498](#), *Nomuraea rileyi*, accession number [AY264288](#), *Metarhizium flavoviride*, accession number [AJ243014](#), *Verticillium fungicola*, accession number [AY292527](#)) from different genera of Hypocreales were selected and aligned using DNASTar to identify conserved regions used to design the degenerate primers chitU (5'-GCCGTCTAC TTCACCAAYTGG-3') and chitD (5'-CCAGCATAGTCGTA GGCCAT-3'). The amplification conditions for the partial sequence of *Vlchit1* were: 5 min at 95°C denaturation, followed by 30 cycles of 94°C for 1 min, 57°C for 1 min and 72°C for 2 min, and a final extension at 72°C for 10 min.

According to the partial sequence of endochitinase gene *Vlchit1* which was amplified with degenerate primers, gene specific primers GSP1 (5'-GCGGCAATAGAAAGCAGGA AATGG-3') and GSP2 (5'-GGCGATACCTATGCGGACTA CGAG-3') were designed for 5' RACE and 3' RACE, respectively. 5' RACE and 3' RACE were conducted by using BD SMART™ RACE cDNA Amplification Kit (Clontech) as recommended by manufacturer.

According to the hypothetical open reading frame (ORF) sequence deduced by analyzing contig assembled with 5' and 3' RACE sequence, primers ORFup (5'-CCGGAATT CATGTTGAGCCTACTCAAAAAA-3') which contained an *EcoRI* site before the ATG codon, and ORFdown (5'-CCCA AGCTTCTATTTTCATGCCATTCTTGAT-3') which had a *HindIII* site after the stop codon were designed for ORF amplification. The total DNA and first strand cDNA were used as template respectively, and the amplification parameters were as follows: 5 min of denaturation at 95°C, followed by 30 cycles of

denaturation for 1 min at 94°C, annealing for 1 min at 50°C, and extension for 2 min at 72°C. An extra extension step consisting of 10 min at 72°C was added after completion of the 30 cycles.

All sequences were cloned to pMD18-t Vector (Takara), transformed into *E. coli* JM109 and sequenced by Takara Bio Company (Dalian, China).

Homology modelling

The deduced amino acid sequence from chitinase gene *Vlchit1* was used in homology modelling. Homology modelling was done using the program Swiss-model (15) and protein structures and models were viewed by software DeepView-Swiss-PdbViewer (<http://swissmodel.expasy.org//SWISS-MODEL.html>).

Production of recombinant overexpression vector

ORF of *Vlchit1* was cloned into the pMAL-c2X overexpression vector (NEB) and fused to the *malE* gene that encodes maltose-binding protein (MBP) to construct recombinant overexpression vectors. The vector pMAL-c2X was double digested with the *EcoRI* and *HindIII*. Amplified ORF of *Vlchit1* were purified from the DNA gel using NucleoTrap® Gel Extract Kit (Clontech) and were digested with *EcoRI* and *HindIII*. Ligation of the insert to the overexpression vector pMAL-c2X vector was performed following the protocol in the NEB technical manual. Transformations of TB1 cells were performed following the protocol in Sambrook *et al.* (14). In-frame insertion of *Vlchit1* with the sequence of MBP in the recombinant clone was selected and verified by digestion analyses with restriction enzyme *EcoRI* and *HindIII* as well as DNA sequencing.

Overexpression and purification of MBP fusion proteins

Selected clones of *Vlchit1* were grown at 37°C in 0.5 L rich broth + glucose & amp (per liter: 10 g tryptone, 5 g yeast extract, 5 g NaCl, 2 g glucose autoclave; add sterile ampicillin to 100 µg/ml) cultures on a rotary shaker. Glucose was added to the growth medium to repress the maltose genes on the chromosome of the *E. coli* host, one of which codes for amylase that can degrade the amylose on the affinity resin that is used for purification. The cells were induced for 4 h with 0.3 mM IPTG (isopropylthiogalactoside) at 37°C when the culture OD₆₀₀ was between 0.4 and 0.6. Both induced and uninduced cells were harvested and resuspended in column buffer (20 mM Tris-HCl (pH 7.4), 200 mM NaCl, and 1 mM EDTA with or without 1 mM DTT) and sonicated. Equal amounts of protein samples of ruptured induced and uninduced cells were loaded on a 10% SDS-polyacrylamide gel and subjected to electrophoresis to verify the overexpression of the recombinant protein.

The fusion protein was purified by one-step affinity chromatography using amylose resin (NEB). Amylose resin (1 ml of amylose resin binds 3 mg of the recombinant protein) was mixed with the crude ruptured cell extract on a shaker at 4 °C for

2 h and poured into a 2.5-cm × 10-cm column to perform batch purification. The column was washed with 1.5 L of column buffer to remove other proteins. At the final step, fusion proteins were eluted from the column by column buffer containing 10 mM maltose.

The protein concentration was determined by using Bradford Method.

Gel electrophoresis

Protein analysis was performed on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Protein samples were reduced by boiling for 5-10 min in loading buffer containing 5% β-mercaptoethanol and then centrifuged at 10,000g for 5min prior to loading the gel. Vertical slab gels, containing 10% (w/v) resolving gel and 5% stacking gel concentrations of acrylamide, were run at a constant current of 15 mA for 6 h. The proteins on PAGE gels were fixed in 45% methanol and 10% acetic acid in distilled water, stained in 0.25% Coomassie brilliant blue R-250 dissolved in 10% acetic acid and 50% methanol in water. The gels were destained for 3 h in 5% methanol and 7% acetic acid in distilled water.

Enzymatic activity assay

Chitinase activity was determined by measuring the reducing end group N-acetylglucosamine produced from colloidal chitin. Reaction mixture consisting 0.5ml enzyme solution, and 0.5ml of 1% (w/v) colloidal chitin (pH 7.0) was cubated at 40°C for 2 h. Termination of the reaction was done by adding 1ml dinitrosalicylic acid reagent and heating in boiling water for 5min. The reaction was then cooled to room temperature, and centrifuged at 10,000g for 10min. The supernatant was subjected to spectrophotometric measurement at 530nm. One unit of chitinase activity was defined as the amount of enzyme that released sugars equivalent to 1 μmol of N-acetylglucosamine per min at 40°C.

RESULTS AND DISCUSSION

Cloning and sequence analysis of the endochitinase gene *Vlchit1*

With the first strand cDNA and degenerate primers, a partial sequence of 688 bp was obtained by PCR amplification. Sequence analysis by NCBI BLAST (<http://www.ncbi.nlm.nih.gov/blast/>) showed that the sequence has high levels of identity with fungal chitinases. Based on the initial sequence gene specific primers GSP1 and GSP2 were designed and a 1567-bp sequence was acquired by 5' and 3' RACE reactions. Sequence analysis indicated that the full length of the chitinase cDNA contained a 95-bp untranslated sequence at the 5' end, a 1272-bp ORF encoding 423-amino-acid endochitinase precursor, and an untranslated region of 200 bp at the 3' end terminating with a poly(A) tail. The sequence of the ORF deduced from the

3' and 5' RACE reactions was confirmed by conducting RT-PCR with primers ORFup and ORFdown. The resulting 1272 bp band was sequenced and shown to be identical to that obtained by RACE-PCR amplification. However, amplification of the genomic DNA with the same primers produced a 1,432-bp band. The overlapping amplified regions above resolved the *Vlchit1* gene (GenBank accession number **DO412944**). The entire sequence is 1,699-bp long with 4 exons and 3 introns (Fig. 1). These introns contain the conserved 5' GT and 3' AG ends.

Analysis of the deduced *Vlchit1* protein sequence using the program SignalP 3.0 Server (1) (<http://www.cbs.dtu.dk/services/SignalP>) predicted a 22 aa signal sequence containing a hydrophobic structure and a signal sequence cleavage site located between aa 22 and 23. Analysis using ProtParam Tool (6) (<http://www.expasy.org/tools/protparam.html>) revealed that the 401 aa mature chitinase (without signal peptide) has a molecular mass of 43.7 kDa, an aliphatic index of 69.18, a grand average of hydropathicity (GRAVY) of -0.371 and is considered to be a stable protein with an instability index of 26.89. Furthermore, the chitinase is an acidic protein with a theoretical pI of 5.55. Two highly conserved regions (SXGG and DXXDXDXE) of the active domain of the family 18 glycosyl hydrolases were identified in the deduced amino acid sequence of *Vlchit1* (Fig. 1), indicating that *Vlchit1* was a member of glycosyl hydrolase family 18. The potential substrate binding site (VMLSIGG) where X of XXXSXGG represents hydrophobic domain was located at aa site 124 and the catalytic active site (FDGIDIDWE) was located at aa 163 site where glutamic acid (Glu171) is the critical residue involved as the proton donor in the catalytic double-displacement mechanism during hydrolysis (12,18).

A BLAST search showed that the deduced amino acid sequence of *Vlchit1* has a high homology to chitinases from other entomopathogenic fungi. The *Vlchit1* showed 69%, 70%, 72%, 74%, 74% and 92% identity to chitinases from *M. anisopliae* (accession number **AAAY32603**), *M. flavoviride* (accession number **CAB44709**), *B. bassiana* (accession number **AAN41260**), *N. rileyi* (accession number **AAP04616**), *Paecilomyces fumosoroseus* (accession number **AAX19146**) and *Isaria farinose* (accession number **ABD64606**), respectively. Compared with other chitinases from *V. lecanii*, it could be found that the amino acid sequence encoded by *Vlchit1* showed 91.7% and 48.1% sequence homology to CHI2 (accession number **AAV98691**) and CHI1 (accession number **AAX56960**) (10) respectively. Although *Vlchit1* displayed significant homology to CHI2, there was obvious difference between their pI values. The deduced pI value of *Vlchit1* is 5.55, indicating *Vlchit1* is an acidic chitinase, whereas CHI2 was predicted to have a pI value of 7.61, which suggests CHI2 belong to the basic chitinase subgroup (10).

Modelling of *Vlchit1* protein

The deduced amino acid sequence of the *Vlchit1* protein was submitted to the automated comparative protein modeling

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ACGCGGGGGCCGATCAGAGACTAGAAAACAGTCCTCCTTTTGGATTCAGAAGGTCCCCTGAAACGTCGTGCTTCCTTCCCTG      80
TTCCGTTCCGACTACA ATG TTG AGC CTA CTC AAA AAA TCG ATG GCT GTA GCC GTG GCC TTG CAG      143
                M L S L L K K S M A V A V A L Q      16
GCG GTC ACG GCT CTG GCT ACG CCA ATC TCC AAC GAG GTT GGC ATT GAG AAG CGC GGT AGT      203
A V T A L A ↓ T P I S N E V G I E K R G S      36
GGC TTC GCA AAT GCT GTT TAC TTC ACC AAC TG GTTTGTTCGACTTTTAATTCTTCAGCTTTCTTCCACA      272
G F A N A V Y F T N W
CTTGATTAACAAAGGAGCAG G GGC ATC TAC GGC CGT AAC TTC CAG CCC GCA GAC CTT CCA GCT TCG      338
                G I Y G R N F Q P A D L P A S      62
GAG ATC ACC CAT GTG CTC TAT TCT TTC ATG AAC ATC CGC TCA GAT GGC ACC AT GTAAGTCAT      400
E I T H V L Y S F M N I R S D G T I      80
CCTTCATATAGGCTGAATTACGAAACTGATCAAGGCGAAAAG C TTT TCT GGC GAT ACC TAT GCG GAC TAC      470
                F S G D T Y A D Y      89
GAG AAG CAC TAC CCT GGT GAC T GTAAGAAGCCCCACAATTCTTTTGGTATAAGTGAATCACTAACATGCTTTA      543
E K H Y P G D S
G CC TGG AAT GAT GTG GGA AAT AAT GCT TAC GGA TGC GTT AAG CAG CTT TAT TTG CTC AAG      603
    W N D V G N N A Y G C V K Q L Y L L K      116
AAG CAA AAC CGC AAC TTG AAA GTT ATG CTG TCG ATT GGT GGT TGG ACC TGG TCC ACC AAC      663
K Q N R N L K V M L S I G G W T W S T N      136
TTT CCC GCT GCT GCC AGC TCT GCT ACC AGC CGA AAG ACT TTC GCT CAA TCT GCC GTT GGT      723
F P A A A S S A T S R K T F A Q S A V G      156
TTC ATG AAG GAT TGG GGA TTC GAT GGT ATC GAC ATC GAT TGG GAG TAC CCT GCG GAT GCT      783
F M K D W G F D G I D I D W E Y P A D A      176
ACG CAG GCT CAG AAC ATG GTC CTC TTG CTC CAG GCC GTC CGC GAC GAG CTC GAC TCT TAT      843
T Q A Q N M V L L L Q A V R D E L D S Y      196
GCC TCG CAG TAT GCC AAA GGC CAC CAT TTC CTG CTT TCT ATT GCC GCC CCC GCT GGA CCT      903
A S Q Y A K G H H F L L S I A A P A G P      216
GAC AAC TAC AAC AAG CTG AAG CTC GCC GAT CTT GGA AAG GTT CTT GAC TAC GTT AAC CTG      963
D N Y N K L K L A D L G K V L D Y V N L      236
ATG GCC TAC GAC TTT GCC GGC TCG TGG AGT AAC TTC ACC GGC CAC GAC GCC AAC ATG TAC      1023
M A Y D F A G S W S N F T G H D A N M Y      256
GCC AGC AAG GAC AAT GCT AAT GCA ACT CCT TTT AAT ACA AAT GAT GCT GTT CAG GCG TAC      1086
A S K D N A N A T P F N T N D A V Q A Y      276
ATC AAA GGA GGT GTC CCT GCC AGC AAA ATT GTC CTG GGT ATG CCC ATC TAC GGT CGG TCT      1143
I K G G V P A S K I V L G M P I Y G R S      296
TTC GAG AAG ACT GAG GGA ATT GGC AAG CCA TAC AAC GGT ATC GGC TCT GGC AGC TGG GAG      1203
F E K T E G I G K P Y N G I G S G S W E      316
AAC GGT GTA TGG GAT TAC AAA GCT CTT CCC AAG TCT GGG GCC ACC GTC AAG TGC GAC AAC      1263
N G V W D Y K A L P K S G A T V K C D N      336
GCT GTT GTG GGC TGC TAT AGC TAC GAT GCG AGC ACT CAG GAG CTC ATT TCT TTC GAC ACT      1323
A V V G C Y S Y D A S T Q E L I S F D T      356
CCA GCT GTT ATC AAC ACC AAG GTT AGC TGG CTT AAG GGC CAG GGA CTC GGT GGA AGC ATG      1383
P A V I N T K V S W L K G Q G L G G S M      376
TTT TGG GAG GCT TCC GCT GAT AAG AAG GGC GCC GAT TCC CTC ATC AGC ACA AGC CAC CAA      1443
F W E A S A D K K G A D S L I S T S H Q      396
GGT CTT GGT AGC CTG GAC AGC GCT CAG AAC TGC CTT GAC TAC CCC AAC TCA AAG TAT GAC      1503
G L G S L D S A Q N C L D Y P N S K Y D      416
AAC ATC AAG AAT GGC ATG AAA TAG GCACTCAGATATGTTCTGGCTTGATCATTGGCATTGGATCCGTCTTG      1574
N I K N G M K *
CCCTGTACATATTACGCTGCCTGCCCCACTCAAGCTTGACAAAGACTTTCCATACTTTTATTGTACATATTATAGATG      1653
TCACGATCATGTACATAGTCAATTGACTTCACTTTCTTTTTTTCGG      1699

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Figure 1. Nucleotide sequence of endochitinase gene *Vchit1*. The deduced amino acid sequence is shown in one-letter code under the ORF. The three introns are underlined. The putative substrate binding site and catalytic domain of the fungal chitinase are shaded. The cleavage site of the signal peptide is indicated by an arrow.

server (<http://swissmodel.expasy.org/SWISS-MODEL.html>). Five chitinases (**1w9y**, **1w9p**, **1w9u**, **2a3b**, **2a3a**, at the Protein Data Bank <http://www.rcsb.org/pdb/>) with the highest identity to Vlchit1 in amino acid sequence were chosen as modeling templates. The Homology model of the Vlchit1 protein and its active domain are showed in Fig. 2. Homology modeling showed that the Vlchit1 chitinases contain the $(\alpha/\beta)_8$ TIM barrel structure like other members of the class 18 hydrolase family. Several kinds of glycosylhydrolases have a similar $(\alpha/\beta)_8$ TIM barrel structure, which may indicates that this structure may be

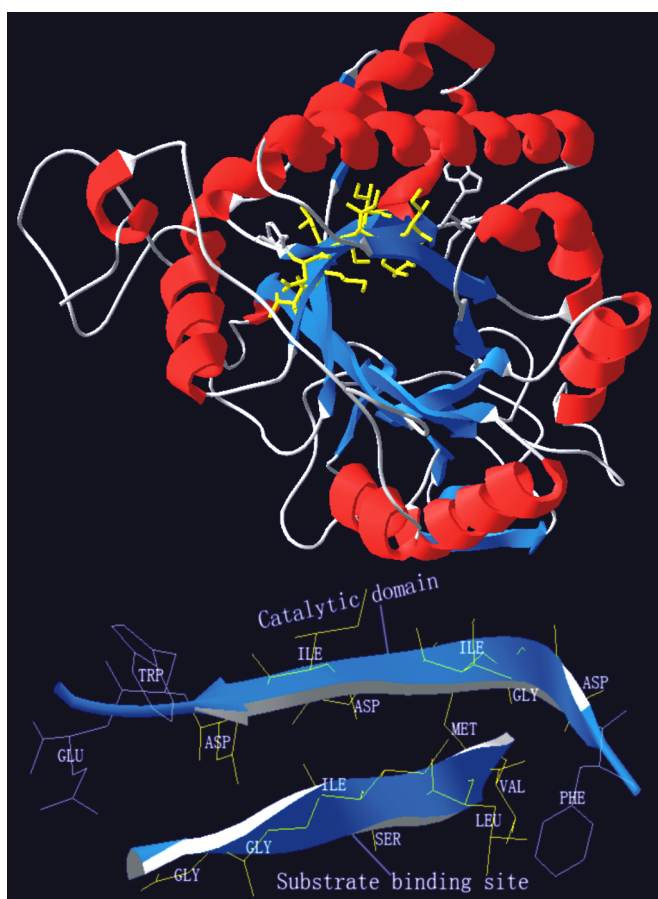


Figure 2. Homology modelling of Vlchit1 protein and its active domain. The SWISS-MODEL program was used to generate the models. (a) Homology model of the Vlchit1 protein, five proteins (**1w9y**, **1w9p**, **1w9u**, **2a3b**, **2a3a**, at the Protein Data Bank <http://www.rcsb.org/pdb/>) with the highest identity in amino acid sequence were chosen as modeling templates. The side chains of putative active domains are shown. (b) Homology model of the putative active domain. The deduced catalytic domain (PHE-ASP-GLY-ILE-ASP-ILE-ASP-TRP-GLU) and substrate binding site (VAL-MET-LEU-SER-ILE-GLY-GLY) are indicated.

important for the hydrolysis of polymeric substrates. Proteins belonging to the class 18 family of chitinases have two conserved regions, as seen in Fig. 1 corresponding to the putative substrate binding site and catalytic domain of Vlchit1. The two signature sequences which lie along barrel strands 3 and 4 of the class 18 chitinases help form the active site cleft on the carboxyl end of the β -barrel and appear to be important both for stability of the fold and for catalytic activity (9,17).

Overexpression and purification of MBP fusion proteins

Preliminary experiment found that *E. coli* cells harboring the recombinant would produce the maximum quantity of MBP-Vlchit1 fusion protein when induced with 0.3 mM IPTG for 4 h at 37°C. The recombinant protein MBP-Vlchit1 was obtained through affinity chromatography purification using amylose resin. The MBP-Vlchit1, total proteins from induced and uninduced cells containing recombinant pMAL-c2X-Vlchit1, total proteins from induced *E. coli* cells harboring vector pMAL-c2X and total proteins from induced *E. coli* cells were loaded on a 10% SDS-polyacrylamide gel and subjected to electrophoresis (Fig. 3). Recombinant protein MBP-Vlchit1 is about 88.4 kDa.

Enzymatic activity assay

Purified recombinant protein MBP-Vlchit1, total proteins from induced cells containing recombinant pMAL-c2X-Vlchit1,

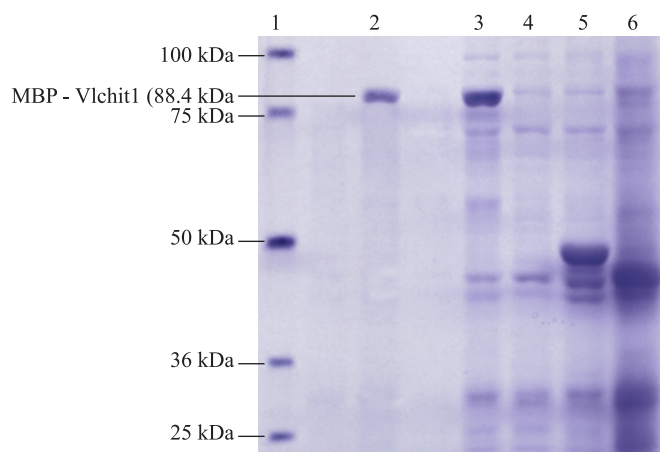


Figure 3. A 10% SDS-polyacrylamide gel showing overexpression of recombinant pMAL-c2X-Vlchit1. Lane 1 represents molecular mass markers. Lane 2 represents purified recombinant protein MBP-Vlchit1. Lanes 3 and 4 represent total proteins from induced and uninduced *E. coli* TB1 cells containing recombinant pMAL-c2X-Vlchit1, respectively. Lane 5 represents total proteins from induced *E. coli* TB1 cells harboring vector pMAL-c2X. Lane 6 represents total proteins from induced *E. coli* TB1 cells.

Table 1. The concentrations and chitinase activities of proteins subjected to chitinase activity assay.

Sample name	Concentration (mg/ml)	Chitinase activity (mU/ml)	Specific activity (mU/mg)
Total proteins from induced <i>E. coli</i> TB1 cells containing recombinant pMAL-c2X-Vlchit1	0.89±0.02	53.1±1.09	59.66±1.23
Total proteins from induced <i>E. coli</i> TB1 cells harboring vector pMAL-c2X	1.27±0.09	0	0
Total proteins from induced <i>E. coli</i> TB1 cells	0.94±0.01	0	0
Purified recombinant protein MBP-Vlchit1	0.39±0.02	47.39±1.11	121.52±2.84

The concentrations, chitinase activities and specific activities are means ± standard deviations of the means based on three replicates.

total proteins from induced *E. coli* cells harboring vector pMAL-c2X and total proteins from induced *E. coli* cells were subjected to chitinase activity assay. Chitinase activity was detected in the purified recombinant protein MBP-Vlchit1 and total proteins of cells containing recombinant pMAL-c2X-Vlchit1, but not in total proteins from induced *E. coli* cells harboring vector pMAL-c2X and total proteins from induced *E. coli* cells (Table 1). The result indicates that the Vlchit1 protein has obvious chitinase activity.

We have cloned the chitinase gene *Vlchit1* from the entomopathogenic fungi *V. lecanii*. The successful overexpression of the *Vlchit1* gene in prokaryotic expression system indicated that Vlchit1 is a functional enzyme that can hydrolyze the chitin substrate. So the *Vlchit1* gene can service as a useful gene source for genetic manipulation leading to strain improvement of entomopathogenic fungi or constructing new transgenic plants with resistance to various fungal and insects pests. Further study on constructing recombinant *V. lecanii* strains which are able to overproduce Vlchit1 chitinase is currently under way in this laboratory.

ACKNOWLEDGEMENTS

This research was supported by grants from Chinese National Natural Science Foundation (30500005), Fujian Provincial Science Foundation for Innovative Young Scholarship (2005J016), Fujian Provincial Educational Programs for Science and Technology Development (JA05239) and Anhui Provincial Key Lab for Microbial Control (2004A04).

RESUMO

Isolamento e caracterização de um gene de quitinase do fungo entomopatogênico *Verticillium lecanii*

O fungo entomopatogênico *Verticillium lecanii* é um agente promissor no controle da mosca-branca e do pulgão. As quitinases secretadas por esse patógeno de insetos têm uma

grande importância no controle biológico de doenças causadas por insetos. Um gene de endoquitinase *Vlchit1* desse fungo foi clonado e expresso em *Escherichia coli*. O gene *Vlchit* contém não apenas um ORF que codifica uma proteína de 423 aminoácidos, mas também é interrompido por três pequenos introns. A modelagem de homologia da proteína Vlchit1 indicou que a quitinase Vlchit1 tem uma estrutura (a/b)₈ TIM barrel. Testes de expressão e de atividade enzimática indicaram que Vlchit1 é uma enzima funcional que hidroliza quitina, portanto o gene *Vlchit* pode ser um gene útil para manipulação genética para melhoramento de cepas de fungos entomopatogênicos ou para a construção de novas plantas transgênicas com resistência a várias doenças causadas por fungos e insetos.

Palavras-chave: quitinase, clonagem, modelagem por homologia, *Verticillium lecanii*

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